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Original Article

DEVELOPMENT OF HERBAL CAPSULES CONTAINING MULBERRY LEAF AND BLACK TEA EXTRACTS USING THE MODIFIED LIQUISOLID TECHNIQUES

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ABSTRACT

Objective: The objective of this study was to develop the capsules containing mulberry leaf extract (MLE) and black tea extract (BTE).

Methods: MLE and BTE were prepared by maceration and determined for phytochemicals, *in vitro* alpha-amylase and alpha-glucosidase inhibitory activities using the enzymatic colorimetric assay. The granules of MLE and BTE were prepared by the application of liquisolid technique and evaluated for the flow properties. The selected granule formulation was filled into the hard gelatin capsule and evaluated for weight variation and disintegration.

Results: The yields of MLE and BTE solid extracts were 8.12 and 4.23% w/w, respectively. Total phenolic and total flavonoid contents were 32.46±5.22 mg TAE/g DW and 44.03±3.37 mg QE/g DW for MLE and 244.66±23.28 mg TAE/g DW and 214.43±3.22 mg QE/g DW for BTE, respectively. The IC_{50} for alpha-amylase of MLE and BTE were 0.69±0.04 and 3.34±0.08 mg/ml, respectively; whereas those for alpha-glucosidase of MLE and BTE were 0.67±0.42 and 0.43±0.15 mg/ml, respectively. The granule prepared with MCC and silica at the ratio of 20:1 showed the highest flowability. The weight variation of the prepared MLE and BTE capsules was within the range of the limitation criteria of ±5%. The average disintegration time of capsules was 1.1±0.1 min.

Conclusion: Herbal capsules of MLE and BTE were successfully prepared. The suitable carrier and coating were MCC and silica with a ratio of 20:1. This study revealed the potential application of liquisolid technique as a tool to produce a capsule of herbal crude extracts.

Keywords: Mulberry leaf extract, Black tea extract, Capsule, Liquisolid.

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INTRODUCTION

Obesity is associated with metabolic disorders, namely hyperglycemia, and hyperlipidemia. The available therapeutic approaches for relieving obesity contain a number of side effects. Therefore, attention to a natural product that is characterized as an anti-obesity agent has been growing [1-3]. The new generations of nutraceuticals, as well as pharmaceutical ingredients that inhibit the breakdown of complex carbohydrate and fats within the gastrointestinal tract have been investigated. Retardation of glucose absorption through the inhibition of carbohydrate-hydrolyzing enzymes, i.e., alpha-amylase and alpha-glucosidase, in the digestive tract is another mechanism causing reduction of blood glucose level, resulting in lower caloric absorption [1].

Mulberry (*Morus alba* L.), a plant in the genus Morus of the family *Moraceae*, has long been used as medicine and food in Asia (China, Japan, and Thailand). Mulberry leaves have potential as a functional food source due to their biologically active compounds, including flavonoids, steroids, amino acids and vitamin [4]. In particular, mulberry leaves contain a large number of iminosugar alkaloids including 1-deoxynojirimycin (1-DNJ), which has strong inhibitory effects on mammalian glucosidase enzymes [1-4]. It was reported that mulberry leaves helped to suppress body weight gain induced by chronic ingestion of a high-fat diet. The major mechanism of which was related to the inhibition of adipogenesis [5]. At present, mulberry leaves are available in the form of dry leaf tea, dry leaf powder (capsules or tablets), and capsules of mulberry leaf extract.

Tea, the most consumed beverage in the world besides water, is made from the leaves of *Camellia sinensis*. Black tea refers to the leaves that have been completely oxidized before desiccation [6]. Tea is an excellent source of polyphenolic compounds, particularly flavonoids. The inhibitory activities of black tea polyphenols against alpha-amylase and glucosidases, as well as glucose transporters, have been demonstrated in many studies. There is increasing evidence that black tea polyphenols have an effect on obesity prevention and management [1]. At present, tea is available in the form of dry leaf tea, dry leaf powder, drinks, as well as capsules or tablets of tea extracts.

A "liquisolid system" refers to formulations formed by conversion of liquid drugs or drug solution in nonvolatile solvents into dry, non-adherent, free-flowing and compactible powder mixtures by blending the solution with selected carriers and coating materials [7]. Liquisolid system offers powders suitable for encapsulation or tableting. In addition to its advantages in terms of simplicity, cost effectiveness and industrial production capability, liquisolid technology had also been used to improve dissolution rates of poorly water-soluble compounds. The enhanced drug dissolution was attributable to increased surface area and aqueous solubility, as well as improved wettability of drug particles [8].

The objective of this present work was to develop the herbal capsules containing mulberry leaf extract (MLE) and black tea extract (BTE). The MLE and BTE were prepared and characterized for their phytochemical contents, antioxidant, alpha-amylase, and alpha-glucosidase inhibitory activities. The herbal capsules containing granules of MLE and BTE were prepared by the application of liquisolid technique, where the volatile solvent, namely hydroethanolic liquid, was used instead of nonvolatile solvents and characterized.

MATERIALS AND METHODS

Materials

The fresh mulberry leaf (*Morus alba*) was collected from a local market in Sakon Nakhon Province, Thailand. A voucher specimen (HB188/61) was identified and kept at the Center for Research and Development of Herbal Health Products, Faculty of Pharmaceutical Sciences, Khon Kaen University. Black tea dry leaf was purchased from LaemthongCoffee shop (Khon Kaen, Thailand). Alpha-amylase from porcine pancreas, alpha-glucosidase from *Saccharomyces*

cerevisiae, p-nitrophenyl-β-D-galactopyranoside (PNPG), 1, 1-Diphenyl-2-picrylhydrazyl hydrate (DPPH), Folin-Ciocaleu reagent and gallic acid were purchased from Sigma (St. Louis, USA). Quercetin was purchased from Fluka (St. Gallen, Switzerland). Microcrystalline cellulose (MCC; Avicel PH102) was provided by Onimax Co., Ltd., Bangkok, Thailand. Dibasic calcium phosphate (DCP; Di-comprez) was obtained from Sudeep Pharma, India. Spray dried α-lactose monohydrate (lactose; FlowLac 100) was supplied by Meggle Group, Wasserburg, Germany. Colloidal silicon dioxide (colloidal silica, Aerosil 200) was purchased from Maxway Co. Ltd (Bangkok, Thailand). All other chemicals and reagents used in this study were of analytical grade.

Preparation and evaluation of mulberry leaf extract (MLE) and black tea extract (BTE)

Preparation of MLE and BTE

MLE and BTE were prepared using the maceration method. For MLE, the fresh mulberry leaves were oven dried (45 °C until constant dried weight obtained) and ground to powder. This dried powder was macerated in 50% ethyl alcohol, using a 1:10 ratio of powder to alcohol, for 7 d with frequent stirring. After filtering through Whatman No.1 paper, the liquid filtrate was collected and evaporated under rotary vacuum evaporator (SB-1000, Eyela, Japan) at 45 °C and then freeze-dried in a lyophilizer (Christ, German). The extraction yield was calculated. In the case of BTE, they were prepared using a similar protocol as MLE, except that the solvent used was 95% ethyl alcohol. The obtained extracts were collected and kept at -40 °C until used.

Phytochemical determination of MLE and BTE

Determination of total phenolic content

Total phenolic content of the extracts was determined using the modified Folin-Ciocalteu method [9, 10]. Briefly, the extract was dissolved in methanol (0.1–5 mg/ml). The extract solution (0.5 ml) was mixed with 0.25 ml of the Folin-Ciocalteau's reagent and 1.25 ml of 20% sodium carbonate solution. The mixture was incubated for 40 min at room temperature. The optical density of the blue-colored samples was measured at 725 nm by a UV–VIS spectrophotometer (Shimadzu, Japan). Tannic acid was used as a calibration standard and the total phenolic content was expressed as mg of tannic acid equivalents/g dry weight of the extract (mg TAE/g).

Determination of total flavonoid content

Total flavonoid content of the extracts was determined using the aluminum chloride ($AlCl_3$) colorimetric method [11]. The extract was dissolved in 50% ethyl alcohol at various concentrations (0.1-5.0 mg/ml), and then the extract solution (1 ml) was mixed with 2% $AlCl_3$ solution (1 ml). After incubation at room temperature for 1 h, the absorbance was measured at 420 nm using a UV–VIS spectrophotometer (Shimadzu, Japan). Quercetin was used to construct the standard curve. The total flavonoid content was expressed as mg of quercetin equivalents/g of dry weight of the extract (mg QE/g).

Antioxidant activities

The free radical-scavenging capacity of the extracts was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical according to a previous report [12]. The extract was dissolved in methanol to make various sample concentrations (1–8 µg/ml). Each 1400 µl of the extract solution was mixed with 100 µl of freshly prepared 1 mmol DPPH in methanol and allowed to stand in the dark for 15 min at room temperature. The absorbance was measured at 515 nm using a UV–VIS spectrophotometer (Shimadzu, Japan). The DPPH free radical-scavenging ability was calculated as half maximal effective concentration (IC_{50}, µg/ml), and obtained by interpolation from the linear regression analysis. Ascorbic acid was used as a positive control.

Enzymatic inhibitory activities of MLE and BTE

Alpha-amylase inhibition

The alpha-amylase inhibitory activity of the extracts was evaluated according to the previous report [13]. A 200 µl of MLE solutions (0.2, 0.4, 0.6, 0.8, and 1.0 mg/ml) or BTE solutions (1, 2, 3, 4, and 5 mg/ml) or acarbose solutions (1, 2, 3, 4, and 5 mg/ml) in 0.2 M potassium phosphate buffer (pH 6.8), were homogeneously mixed with 500 µl of 0.2 % w/v starch and 100 µl of 2 units/ml α-amylase solution in a test tube. After being incubated at 40 °C for 1 h, 500 µl of 4 mg/ml color reagent (3, 5-dinitrosalicylic acid) and 2 ml of 2 M sodium hydroxide were added. The test tube of the mixture was incubated in boiled water for 10 min, and then the solution was removed from the heating process and cooled at room temperature, with the addition of 2700 µl of cold water to stop the enzymatic reaction. The solution was homogenized using a vortex. The α-amylase activity was determined at 540 nm using a UV–VIS spectrophotometer (Shimadzu, Japan) to measure product absorbance (maltose) which reduced dinitrosalicylic acid. The produced absorbance was compared with a blank. Percent inhibition was calculated using equation 1.

$$\text{Inhibition (\%)} = \frac{\left(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}\right)}{\text{Abs}_{\text{control}}} \times 100 \quad \text{..... (1)}$$

Where Abs_{sample} represents absorbance of samples being tested and $Abs_{control}$ represents that of 0.2 M potassium phosphate buffer (pH 6.8).

Alpha-glucosidase inhibition

The alpha-glucosidase enzyme reaction was performed using p-nitrophenyl-β-D-galactopyranoside (PNPG) as a substrate [13, 14] A 100 µl of MLE solutions (0.2, 0.4, 0.6, 0.8, and 1.0 mg/ml) or BTE solutions (1, 2, 3, 4, and 5 mg/ml) or acarbose solutions (1, 2, 3, 4, and 5 mg/ml) in 0.2 M potassium phosphate buffer (pH 6.8) were mixed with a 80 µl of PNPG solution (0.25 mg/ml) in a 96 well microplate, and then incubated at 37 °C for 20 min. After that, a 20 µl of alpha-glucosidase solution (1 unit/ml) in 0.2 M potassium phosphate buffer (pH 6.8) was added in each well to obtain a total volume of 200 µl. The mixture was incubated to get the complete hydrolysis reaction. The absorbance of p-nitrophenol released from PNPG was detected at 405 nm after incubating the mixture at 37 °C for 30 min in Benchmark plus Microplate Spectrophotometer (BIO-RAD Laboratories Ltd., Japan). Percent inhibition was calculated using equation 1. The 50% inhibition of enzymatic activity (IC_{50}) was calculated using the linear regression equation.

Preparation and evaluation of MLE and BTE granules

Preparation of granules

Several MLE and BTE granules, symbolized as CN, LS1-LS5 (table 3) were prepared by the application of liquisolid technique. For conventional (CN) granules, MCC was used as the adsorbent. In the case of liquisolid (LS), the adsorbent composed of carrier and coating, of which MCC, DCP or lactose and colloidal silica were used as carrier and coating material, respectively.

The MLE and BTE were first dissolved using 80% ethyl alcohol in water, with the ratio of extracts to solvent of 1:50. The MLE and BTE hydroalcoholic solution was gradually added onto the adsorbent powder (MCC for CN and admixture of carrier and coating for LS formulations) with the application of continuous mixing in a glass mortar. The obtained damp mass was passed through sieve #16, ambient dried for 15 min, and subsequently oven-dried at 50 °C for 30 min. The dry granules were passed through sieve #18 before being kept in a light-resistant container. The obtained granules were kept in a desiccator until used.

Evaluation of granules

Loss on drying

Loss on drying (LOD) test was performed in accordance with the method elaborated in pharmacopeia [15] using Sartorius Thermo Control (YTC01L, Sartorius, Germany). It was calculated as the percentage of weight loss (% w/w) resulting from water and volatile matter of any kind driven off under specified conditions.

Flow properties

Two techniques were used to evaluate the flow properties of powders: Carr's compressibility index (CI) and flow rate.

CI was calculated using tapped and bulk densities. A weighed quantity of the prepared powder admixture was carefully poured into 100 ml graduated cylinders. The volume occupied by the

powder was read, and the bulk density was calculated in g/ml. The tapped density was calculated using tapped volume which was obtained after sufficient taps. CI of each powder mix was calculated using equation (2) [16].

$$CI\ (\%) = \frac{(\text{Tapped density - Bulk density})}{\text{Tapped density}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

The granule flow rate was determined using a flow through an orifice (glass funnel) method [15]. Briefly, a glass funnel was secured with its tip positioned at a given height above a flat horizontal surface. The powder flow rate as the mass per time flowing from a funnel was calculated.

Preparation and evaluation of herbal capsules of MLE and BTE

Preparation of herbal capsules

Herbal capsules containing 30.0 mg of MLE and BTE each were prepared in batches of 100 capsules using semiautomatic capsule filling machine (Charatchai Machinery, Thailand). Approximately 210 mg of the selected MLE-BTE granules were filled into hard gelatin capsules (size 1).

Evaluation of herbal capsules

Weight variation

The weight variation was determined through random selections of twenty capsules from each batch. The selected intact capsules were then weighed individually and accurately using an electronic balance (GF-600, AND, Japan), and the average weight was determined.

Disintegration time

The disintegration test was performed at 37±0.5 °C in distilled water for six capsules from each formulation, using a basket-rack assembly disintegration test apparatus (Model QC-21, Hanson Research, Northridge, CA). The average disintegration time was calculated.

Statistical analysis

The statistical analysis was performed using the SPSS program for Microsoft Windows, release 19 (SPSS (Thailand) Co. Ltd., Bangkok, Thailand). The results were expressed as the mean±SD. One-way ANOVA and independent samples t-test were used to test the statistical significance of differences among groups. The significance was determined with 95% confidence limits (α=0.5) and was considered significant at a level of P less than 0.05.

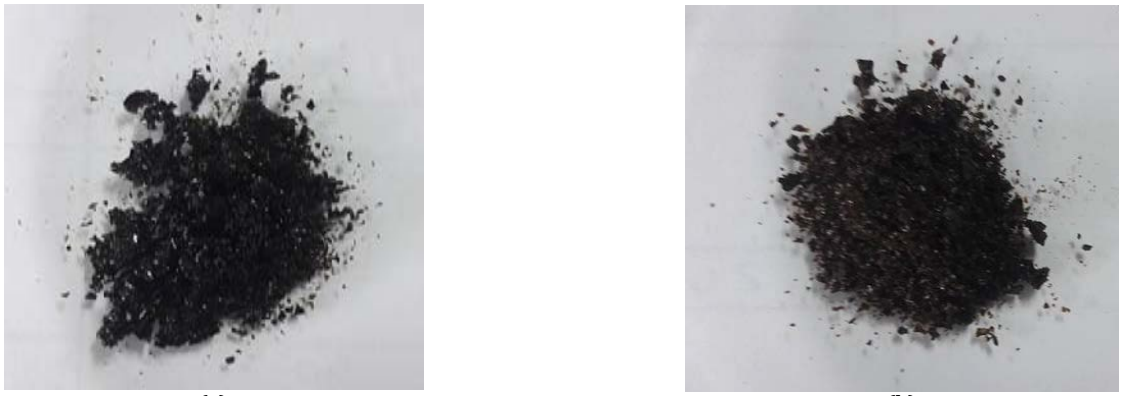
RESULTS AND DISCUSSION

Several biological properties of mulberry leaves have been reported, including antihyperglycaemic, antihyperlipidaemic, anti-obesity, antihypertensive, antioxidative, anti-inflammatory, anti-atherosclerosis, and cardioprotective effects. The dominant compounds responsible for the pharmacological effects of mulberry leaves included DNJ, phenolics, and flavonoids [5, 17]. 1-DNJ is the most dominant iminosugar alkaloid in mulberry leaves which exhibits an inhibitory effect on mammalian glucosidase enzymes. Due to the high polarity of DNJ, solvent toxicity and consumer safety consideration, a mixture of ethanol and water (50% ethanol) was used as the solvent in this study.

The hypoglycaemic, antihyperglycaemic and antidiabetic potential of black tea had been reported. The polyphenols present in the tea are mainly ascribed for these pharmacological effects of tea [18]. Numerous studies also revealed the anti-obesity effects of black tea polyphenols in animals. It was reported that the highest polyphenol content-tea extract was obtained with 95% ethanol [19].

Therefore, the present study, BTE was prepared using 95% ethanol.

MLE was dark green solid extract while BTE was dark brown-green solid extract as seen in fig. 1. Both of the prepared extracts were very hard and difficult to resize. Additionally, the hygroscopicity of such freeze-dried extracts made them sticky and difficult to flow. The yield of MLE was 8.12 %w/w, whereas that of BTE was 4.23% w/w (table 1).



(a) **(b)**

Fig. 1: The mulberry leaf extract (MLE) (a) and black tea extract (BTE) (b)

Table 1: Yield, total phenolic, and total flavonoid contents and antioxidant (DPPH) of mulberry leaf extract (MLE) and black tea extract (BTE)

Samples	Yield (%)	Total phenolic (mg TAE/g DW)	Total flavonoid (mg QE/g DW)	DPPH (IC_{50} µg/ml)
MLE	8.12	32.46±5.22	44.03±3.37	331.68±13.07
BTE	4.23	244.66±23.28	214.43±3.22	108.07±0.11

TAE/g DW: tannic acid equivalent per gram dried weight, QE/g DW: quercetin equivalent per gram dried weight, mean±SD, n = 3.

Phytochemicals of MLE and BTE

The determined phytochemical compounds in MLE and BTE, consisting of total phenolic and total flavonoid contents, is presented in table 1. The determination of total phenols was accomplished using the Folin-Ciocalteu method. Results were expressed in mg of tannic acid/g of dry matter. The flavonoid contents were determined by a spectrophotometric method based on the complex formation with aluminum chloride. Results were expressed in mg of quercetin/g of dry matter.

It was found that total phenolic and total flavonoid contents of MLE were 32.46±5.22 mg TAE/g DW and 44.03±3.37 mg QE/g DW, respectively. These findings were consistent with the previous

studies. The wide range of total phenolic contents of mulberry leaves and extract, i.e., 7.9 to 260.0 mg gallic acid equivalent/g DW, has been reported [20-23]. The total flavonoid contents of mulberry leaves and extract reported ranged from 9.8 to 33.3 mg rutin equivalent/g DW [20, 22, 24].

In the case of BTE, total phenolic and total flavonoid contents were found to be 244.66±23.28 mg TAE/g DW and 214.43±3.22 mg QE/g DW, respectively. The levels of total phenols obtained in this study were consistent with those reported in the literature. It was reported that the total phenolic contents of tea extract ranged from 245.8 to approximately 600 mg catechin equivalent/g DW, depending on extraction solvents [19]. The total flavonoids of tea extract varied in the range of 6.1 to 47.4 mg quercetin equivalent/g DW [25, 26].

In this study, the total phenolic content of BTE was significantly higher than that of MLE (*P<0.05). The total phenolic content of BTE was approximately 7.5 times higher than that of MLE. Similar to the total phenolic content, the total flavonoid content of BTE was significantly higher than that of MLE (*P<0.05), or approximately 5 times higher. However, other researches on total phenolic and total flavonoid contents in leaves and extracts of mulberry and tea showed different results. It has been known that the range of chemical compositions in the leaves can considerably vary among different samples. The variety, growing environment, harvesting seasons, manufacturing conditions, grade (particle size) of the leaves, extraction solvents and conditions, as well as concentration procedures, are the factors influencing the final extract compositions.

Antioxidant activities of extracts

In this study, the antioxidant activities of both extracts were determined using DPPH radical scavenging activity and shown in table 1. The DPPH radical scavenging assay is one of the most popular techniques to determine the antioxidant activity potential of any plant material due to its stability and simplicity [27]. The ability of extracts to scavenge the DPPH radical was expressed as IC_{50} value, i.e., the concentration required to inhibit radical formation by 50%, where a lower value of IC_{50} indicated a higher antioxidant activity.

It was found that both MLE and BTE demonstrated antioxidative effects in a dose-dependent fashion. The IC_{50} of MLE was 331.68±13.07 µg/ml, whereas that of BTE was 108.07±0.11 µg/ml. However, the effect was much weaker than that of ascorbic acid which was used as the positive control (IC_{50} = 2.26±0.11 µg/ml). The ability of mulberry and tea leaves to neutralize free radicals was confirmed by several evaluation methods. Nevertheless, differences in plant sources and extraction solvents resulted in different antioxidant activities. The reported IC_{50} of mulberry extracts varied from 12.4 to 815.3 µg/ml [22, 28]; whereas those of tea extracts were in the range of 210.7 to 390.1 µg/ml [19].

The antioxidant activity of BTE was approximately 3 times higher than that of MLE (*P<0.05). It has been known that phenolics and flavonoids are excellent antioxidants. The relatively stronger antioxidative property of BTE compared to that of MLE may be due to the higher values of phenolic and flavonoid compounds of the extract.

Black tea is a rich source of polyphenols, which showed potent antioxidant activities both *in vitro* and *in vivo*, including catechins, theaflavins, and thearubigens [29]. Theaflavins are formed by polymerization of catechins at the fermentation or semifermentation stage during the manufacture of black tea. The major theaflavins found in black tea are theaflavin, theaflavin digallate, theaflavin monogallate A and theaflavin monogallate B. Previous studies have shown that catechins and theaflavins are equally effective antioxidants, with strong free radical–scavenging activity both *in vitro* and *in vivo* [30]. Different forms of catechins found in tea leaves were epigallocatechin, epicatechin, epicatechin gallate, and epigallocatechin gallate. Nevertheless, the most abundant form of catechin in black tea was epigallocatechin gallate [31]. In addition to being potent antioxidants, these polyphenols present in the tea are mainly ascribed for its pharmacological effects [30].

The ability of mulberry leaves to prevent free radical formation and oxidative stress induced tissue damage was confirmed by several evaluation methods. A number of antioxidative compounds of mulberry leaves were isolated and identified. Phenolic compounds found in mulberry leaves were chlorogenic acid, caffeic, gallic, ferulic, protocatechuic, p-hydroxybenzoic, p-coumaric, m-coumaric, vanillic, syringic, and sinapic acids [21]. The phenolics in mulberry leaves were hypothesized to be responsible for the anti-obesity effects of mulberry leaves [32]. The flavonols fraction contained rutin (3-O-rutinoside quercetin), isoquercitrin (quercetin 3-β-D-glucoside) and astragalin (kaempferol 3-β-D-glucopyranoside) [21].

Enzyme inhibitory activities

The ability of MLE and BTE to decrease saccharide digestion and absorption through inhibitory activities against alpha-amylase and alpha-glucosidases was investigated.

Dietary carbohydrates were degraded into disaccharides, such as maltose and sucrose, using α-amylase [33]. The effects of MLE and BTE on alpha-amylase activity in the *in vitro* digestion process of dietary carbohydrates were investigated and shown in table 2

Table 2: Inhibitory effects of MLE and BTE on alpha-glucosidase and alpha-amylase enzymes

Sample	alpha-glucosidase (IC_{50} mg/ml)	alpha-amylase (IC_{50} mg/ml)
MLE	0.67±0.42	0.69±0.04
BTE	0.43±0.15	3.34±0.08
Arcabose	1.35±0.01	0.29±0.02

mean±SD, n = 3.

It was found that the IC_{50} for alpha-amylase of MLE was significantly lower than that of BTE (0.69±0.04 mg/ml and 3.34±0.08 mg/ml, respectively) (*P<0.05). These results indicated that MLE showed greater alpha-amylase inhibitory activities compared to BTE. However, IC_{50} values of both extracts were significantly higher than that of acarbose (0.29±0.02 mg/ml) (*P<0.05) which was used as the positive control, indicating that both extracts had lower alpha-amylase inhibitory activities compared to acarbose.

The IC_{50} for alpha-amylase of MLE in this study was in agreement with the previous studies which reported that the IC_{50} values of mulberry leaf extracts were less than 1 mg/ml to 17.60 mg/ml [34, 35]. Previous studies reported the IC_{50} values for alpha-amylase of black tea extract to be in the range of 1.74 mg/ml to 2.90 mg/ml [33, 36]. The IC_{50} of BTE obtained in this study was higher than the value reported in the literature. This might relate to the differences in extract compositions in terms of the variety, growing environment, harvesting seasons, manufacturing conditions, extraction solvents, and conditions, as well as concentration procedures.

Alpha-glucosidase is an enzyme in small intestine responsible for digestion of disaccharides, such as maltose and sucrose, into monosaccharides, glucose, prior to absorption [33]. The effects of MLE and BTE on alpha-glucosidase activity were investigated *in vitro* and the results are shown in table 2.

It was found that IC_{50} values on alpha-glucosidase inhibition of MLE and BTE were 0.67±0.42 mg/ml and 0.43±0.15 mg/ml, respectively. These results indicated that the alpha-glucosidase inhibitory activities of BTE and MLE in this study were comparable (P>0.05). Furthermore, these IC_{50} values suggested that both extracts were more potent than that of acarbose, a positive control, on inhibition of alpha-glucosidase (IC_{50} = 1.35±0.01 mg/ml) (*P<0.05).

The IC_{50} for alpha-glucosidase inhibition of MLE obtained in this study was in agreement with previous reports [34, 37] which revealed that the IC_{50} values of mulberry leaf extract were in the range of 0.23 to 0.63 mg/ml, depending on extraction solvent. A wide range of alpha-glucosidase inhibitory activities of black tea leaf and extract have been reported, from less than 1 mg/ml to 2.1 mg/ml [38, 39]. The IC_{50} of BTE in this study was higher than those found in the previous studies. The use of different plant sources and extraction protocols might be the reason for the differences in extract compositions.

Morus alba (mulberry) leaves have been used in traditional medicine for the treatment of diabetes mellitus [40]. It has been reported that the compounds found in mulberry leaf extract, including chlorogenic acid, 1-DNJ, rutin, isoquercitrin, astragalin, caffeic acid, quercetin, and quercitrin, as well as chlorogenic acid, exhibited relatively stronger alpha-amylase inhibitory activities compared to other compositions [41]. Chlorogenic acid played an important role in the overall alpha-amylase inhibitory activity of mulberry leaf extract; whereas iminosugars, namely 1-DNJ and N-methyl-1-deoxynojirimycin (N-methyl-1-DNJ) were the most dominant compounds responsible for the strong actions on alpha-glucosidase inhibition [42, 43]. 1-DNJ and its derivatives have been found to competitively block the active site of polysaccharide-degrading enzymes in the digestive tract due to the similar structures of DNJ to glucose, i.e., when the enzymes are inhibited, digestion and absorption of dietary carbohydrates will be eventually diminished. A recent study revealed that other flavonoids found in mulberry leaf extract, namely rutin, isoquercitrin, kaempferol-3-O-rutinoside, and astragalin, also showed alpha-glucosidase inhibitory activity [42].

Several studies have demonstrated that the inhibitory activities of black tea against alpha-amylase and glucosidases are due to its polyphenols. Phenolic compounds are known to block nucleophilic sites of digestive enzymes by binding them to amino acid side chains, which could inhibit alpha-amylase activity. According to a previous report [44], theaflavins, the major phenolic compounds found in black tea exhibited strong alpha-amylase inhibitory activity, and these compounds included theaflavin digallate, theaflavin monogallate A, theaflavin monogallate B and theaflavin. Similarly, catechins (namely epicatechin gallate, epigallocatechin gallate, catechin gallate, and gallocatechin gallate), the minor phenolic compounds in black tea also showed alpha-amylase inhibitory activity. On the contrary, free catechins (namely, epicatechin and epigallocatechin) and their isomers (catechin, gallocatechin), as wel1 as gallic acid did not have significant effects on the activity of alpha-amylase. The components of black tea responsible for alpha-glucosidase activity might include theaflavins, especially theaflavin digallate and catechins (epicatechin gallate and epigallocatechin gallate) [45, 46]. Moreover, Jeon *et al.* [47] reported that the IC_{50} on the alpha-glucosidase activity of theaflavins containing mono-and di-gallate extracted from black tea was very low. The results indicated that the mixed theaflavin components exhibited a stronger inhibitory effect compared to a single component.

Acarbose is the well-known inhibitor of alpha-glucosidase and pancreatic alpha-amylase with antihyperglycemic activity. The excessive alpha-amylase inhibition of acarbose was found to be the cause of abnormal bacterial fermentation of undigested carbohydrates in the large intestine [20, 48]. Consequently, this drug was revealed to be associated with gastrointestinal side effects such as flatulence and abdominal bloating [49]. It was further suggested that plant-based ingredients exhibited lower inhibitory effect against alpha-amylase activity but stronger activity against alpha-glucosidase, indicating that they might be effective agents for the control of postprandial hyperglycemia with fewer side effects compared to acarbose [50].

Preparation of MLE and BTE granules by the application of the liquisolid technique

Similar to other crude extracts obtained from lyophilization, MLE and BTE are dry but sticky extracts. The uniformity in granules of MLE and BTE cannot be achieved through the reduction of particle size and dry mixing with other diluents. Liquisolid system offers powders suitable for encapsulation or tableting with its effective adsorbent systems, namely carrier and coating materials [7, 8]. In case of conventional liquisolid, liquid medicaments which refer to oily liquid or solutions or suspensions of compounds in nonvolatile liquids are the conversion of the liquid into dry, nonadherent, free-flowing powder mixtures. In this study, the volatile hydroalcoholic liquid was used as a solvent for MLE and BTE. The compositions of granule formulation were shown in table 3. The conventional (CN) and liquisolid (LS) granules, symbolized as CN and LS1 to LS6 (table 1) were prepared.

Table 3: Formulation compositions and flow characteristics of MLE and BTE granules

Rx	MLE (mg)	BTE (mg)	Carrier, Q (mg)			Coating, q (mg)	*R*	Unit dose (mg)	L_f	Carr's index	Flow rate (g/s)
			MCC	DCP	Lactose						
CN	30	30	150	-	-	-	-	210	0.40	26.1±1.1	2.3±0.1
LS1	30	30	142.86	-	-	7.14	20	210	0.42	22.4±0.2	3.4±0.0
LS2	30	30	-	142.86	-	7.14	20	210	0.42	35.5±1.0	2.7±0.1
LS3	30	30	-	-	142.86	7.14	20	210	0.42	37.2±1.5	2.4±0.1
LS4	30	30	145.16	-	-	4.84	30	210	0.44	22.2±1.0	3.3±0.0
LS5	30	30	136.37	-	-	13.63	10	210	0.44	22.9±3.2	3.0±0.1

MLE: mulberry leaf extract, BTE: black tea extract, MCC: microcrystalline cellulose, DCP: dibasic calcium phosphate. mean±SD, n = 3, It was found that all of the prepared granules were green-brown, with % LOD of less than 5%. The examples of MLE and BTE granules were shown in fig. 2.

Fig. 2: MLE and BTE granules prepared from formula LS1

Powder flowability is critical to the success of various pharmaceutical processes including capsule filling. In order to achieve uniform particle packing and a constant volume-to-mass ratio, which maintains the weight uniformity, the flow of powders or granules to be filled need to be proper [51]. The effects on granule flow properties of different carriers were studied in formulas LS1-LS3 where MCC, DCP, and lactose were used, respectively. The results of Carr's index showed that LS1 had passable flow property, whereas formulas LS2 and LS3 had very poor flow property. The flow rate of formula LS1 was significantly faster than that of formulas LS2 and LS3 (*P<0.05). The results clearly showed that granules prepared with MCC had better flow properties compared to other carriers.

Carrier and coating selections are the two components crucial for obtaining the optimal liquisolid formulation. For a material to be considered a good carrier, it should have a large surface area in

order to absorb/adsorb a large amount of liquid medication, and offer good flow properties, while retaining the liquid medication and compressibility [52, 53]. According to Spireas *et al.* [54], a carrier should be a particle with a porous surface which allows higher absorption. The adsorptive materials in this study were semipolar extracts. It is known that DCP and MCC are water-insoluble fillers, while lactose is water-soluble filler.

The lactose binding capacity of MLE and BTE (capacity of powder excipients to hold liquid while maintaining their flow properties) might be the lowest due to its hydrophilic property. The binding capacity of MCC might be the highest due to its smaller size compared to DCP and porous surface, which gives it a larger specific surface area. The result of this study was in line with other studies [55, 56]. In fact, MCC has been the most commonly employed carrier particle in liquisolid systems. It was reported that the porous structure of MCC enabled it to absorb a large amount of liquid [57]. Therefore, MCC was selected as the carrier material for MLE and BTE liquisolid granules.

The effects of the different carrier to coating (MCC: silica) ratios at 30:1, 20:1 and 10:1 on granule flow properties was studied in formulas LS4, LS1 and LS5, respectively. The results of Carr's index showed that all formulas had passable flow properties. The flow rate of formula LS1 at the ratio of 20:1 was significantly faster compared to the formulas LS4 at the ratio of 30:1 and LS5 at the ratio of 10:1 (*P<0.05). Furthermore, the granules without coating (formula CN) were also prepared and evaluated for flow properties. The results from Carr's index indicated that formula CN had poor flow property. The flow rate of formula CN was significantly lower than that of all formulas with the coating (*P<0.05). Silica is a substance with a large surface area (201±7 m2/g) and high adsorptive properties. The high flowability of silica can impart the desired flow characteristics of the prepared powders [58].

Based on these results, MCC was used as the carrier in granule formulas. The coating must be used in granule preparation. The carrier to coating (MCC: silica) ratio of 20:1 was used for further studies.

MLE and BTE capsules

The granule formula LS1 was selected for preparation of MLE and BTE capsules. The prepared capsules were evaluated for weight variation and disintegration. The picture of MLE and BTE capsules was shown in fig. 3.

It was found that the average weight of the capsules was 214.6±7.0 mg (n = 20), which was within the range of the limitation criteria of ±5%. The average disintegration time of capsules was 1.1±0.1 min (n = 6), which was within the range of the limitation criteria of 30 min. These results were found to be satisfactory and within the acceptance criteria in pharmacopeia [15].

Fig. 3: MLE and BTE capsules prepared from formula LS1

CONCLUSION

In this study, the herbal capsules of MLE and BTE, which are good sources of unique phytochemical compounds with strong antihyperglycaemic, antioxidative as well as anti-obesity effects, were successfully prepared using the application of liquisolid techniques for the first time. The MLE and BTE were prepared using maceration mulberry and black tea leaves with 50% and 95% ethanol. The total phenolic and total flavonoid contents, antioxidant, inhibitory activities against alpha-amylase and alpha-glucosidases of MLE and BTE were confirmed. The optimal formulations of MLE and BTE granules were prepared using MCC and silica with the ratio of 20:1 as the carrier and coating materials. Weight variation and disintegration tests confirmed that the capsules filled with MLE and BTE granules were within the acceptance criteria. Overall, this study revealed the potential application of liquisolid technique as a tool to produce a capsule of herbal crude extracts. Further studies on the standardization, dissolution, and stability of MLE and BTE polyphenols are to be conducted.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally

CONFLICT OF INTERESTS

The authors report no conflicts of interest

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